

CLAIMS

What is claimed is:

1. A trait removal construct comprising:
 - a) A first recombinase element having the general structure P1-R
 - 5 and;
 - b) A second recombinase element selected from the group of general structures consisting of K-TG, and RS-K-TG-RS;

wherein,

 - (i) P1 is a first promoter;
 - (ii) R is a recombinase coding sequence and 3' region
 - 10 (iii) TG is a transgene;
 - (iv) RS is a recombinase site responsive to the recombinase
 - (v) K is selected from the group consisting of;
 - 1) P2-RS-STP-RS and
 - 15 2) P2

wherein P2 is a second promoter, RS is a recombinase site responsive to the recombinase and STP is a stop fragment;

wherein P1 and P2 are operably linked to their down stream elements and wherein P1 is activated prior to P2 in the plant life cycle and wherein

 - 20 expression of the recombinase results in excision of any element contained between the recombinase site responsive to the recombinase.
2. A trait removal construct comprising:
 - a) A first recombinase element selected from the group of general structures consisting of: P1-R1 and P1-R2, and;
 - 25 b) A second recombinase element having the general structure RS2-K-TG-RS2;

wherein,

 - (i) P1 is a first promoter;
 - (ii) R1 is a first recombinase coding sequence and 3' region;
 - 30 (iii) R2 is a second recombinase coding sequence and 3' region;
 - (iv) RS2 is a second recombinase site responsive to the second recombinase;
 - (v) TG is a transgene sequence and 3' region ;
 - (vi) K is selected from the group consisting of:
 - 35 1) P2-RS1-STP-RS1 and
 - 2) P2-RS1-TG

wherein P2 is a second promoter, RS1 is a first recombinase site responsive to the first recombinase, STP is a stop fragment and TG is a transgene sequence and 3' region ;

wherein P1 and P2 are operably linked to their down stream elements,
5 and wherein P1 is activated earlier than P2 in the plant life cycle, and wherein expression of the first recombinase results in excision of any element contained between the first recombinase sites responsive to the first recombinase and expression of the second recombinase results in excision of any element contained between the second recombinase sites responsive to the second recombinase.

10 3. A trait removal construct comprising:

- a) a first recombinase element selected from the group of general structures consisting of P1-R1 and RS2 -P1-R1 -RS2 ;
- b) a second recombinase element selected from the group of general structures Z-Y and RS2 -Z-Y-RS2 ;
- 15 c) a third recombinase element selected from the group of general structures Q-X and RS2 -Q-X-RS2 ;

wherein:

- (i) P1 is a first promoter;
- (ii) R1 is a first recombinase coding sequence and 3' region;
- 20 (iii) RS2 is a second recombinase site responsive to a second recombinase;
- (iv) Z has the general formula, P2-RS1-STP-RS1, wherein P2 is a second promoter, RS1 is a first recombinase site responsive to a first recombinase, and STP is a stop fragment;
- 25 (v) Y is selected from the group consisting of R2 and TG wherein R2 is a second recombinase coding sequence and 3' region and TG is a transgene sequence and 3' region ;
- (vi) Q has the general formula P3-RS-STP-RS, wherein P3 is a third promoter, RS is a recombinase site selected from the group consisting of RS1 and RS2 , and STP is a stop fragment;
- 30 (vii) X is selected from the group consisting of TG and R wherein TG is a transgene sequence and 3' region and R is a recombinase coding sequence and 3' region selected from the group consisting of R1 and R2;

35 wherein P1, P2 and P3 are operably linked to their down stream elements and wherein P1 is activated earlier than P2 in the plant life cycle, and wherein P2 is activated earlier than P3 in the plant life cycle, and wherein expression of the first recombinase results in excision of any element contained between the first

recombinase sites responsive to the first recombinase and expression of the second recombinase results in excision of any element contained between the second recombinase sites responsive to the second recombinase.

4. A trait removal construct according to Claim 1, 2 or 3 wherein the first
5 promoter is a germline promoter.

5. A trait removal construct according to Claim 4 wherein the germline promoter is selected from the group consisting of:

- a) constitutive plant promoters;
- b) plant tissue-specific promoters;
- 10 c) plant developmental stage-specific promoters;
- d) inducible plant promoters;
- e) viral promoters;
- f) male germline promoters;
- g) female germline promoters;
- 15 h) common germline promoters;
- i) floral common germline promoters;
- j) vegetative shoot apical meristem promoters; and
- k) floral shoot apical meristem promoters.

6. A trait removal construct according to Claim 5 wherein the male
20 germline promoter is derived from genes selected from the group consisting of genes specific to anther primordia and anther sporophyte and to pollen gametophyte.

7. A trait removal construct according to Claim 5 wherein the common
germline promoter is derived from genes selected from the group consisting of
25 *Apetala 3* (AP3), *Pistillata* (PI), synthetic anther promoter, TA29, and BCP1 and orthologs thereof.

8. A trait removal construct according to Claim 5 wherein the common
germline promoter is derived from genes selected from the group consisting
vegetative and floral shoot apical meristems.

9. A trait removal construct according to Claim 8 wherein the common
30 germline promoter is derived from genes selected from the group consisting of *Leafy* (LFY), *Apetala 3* (AP3), *Pistillata* (PI), *Apetala 1* (AP1), *Agamous* (AG) and *Pistillata* (PI) and orthologs thereof.

10. A trait removal construct according to Claim 5 wherein the floral
35 common germline promoter is derived from genes selected from the group consisting of *Agamous* (AG), *Apetala 1* (AP1), *Apetala 3* (AP3), *Leafy* (LFY) and orthologs thereof.

11. A trait removal construct according to Claim 5 wherein the vegetative shoot apical meristem promoter is selected from the group consisting of *Agamous* (AG), *Apetala 1* (AP1), *Apetala 3* (AP3), *Leafy* (LFY), *Aintegumenta* (ANT), *Clavata 3* (CLV3), *Wushel* (WUS), and *Meristemless* (STM) and orthologs thereof.
12. A trait removal construct according to Claim 1, 2 or 3 wherein the second promoter is selected from the group consisting of:
- constitutive plant promoters;
 - plant tissue-specific promoters;
 - plant development stage-specific promoters;
 - inducible plant promoters; and
 - viral promoters.
13. A trait removal construct according to Claim 1, 2 or 3 wherein the transgene is selected from the group consisting of:
- genes encoding a transformation marker;
 - genes encoding a morphological trait;
 - genes conveying sterility;
 - genes conveying specific phenotype on a plant or plant cell; and
 - hormone biosynthetic genes
14. A trait removal construct according to Claim 13 wherein the genes encoding a transformation marker are selected from the group consisting of:
- antibiotic resistance genes;
 - herbicide resistance genes;
 - green fluorescent protein genes;
 - luciferase genes;
 - tumorigenic genes;
 - shoot inducing genes
 - root inducing genes; and
 - selectable genes.
15. A trait removal construct according to Claim 13 wherein the genes encoding a morphological trait are selected from the group consisting of:
- rol C,
 - IPT,
 - Ti plasmid genes,
 - Ri plasmid genes,
 - STM,
 - KNAT,
 - AINTEGUMATA,

- h) *Lec1*,
 - i) *Brassica "Babyboom"*
 - j) *OSHI*, and
 - k) *Kn1*
- 5 16. A trait removal construct according to Claim 13 wherein the genes encoding hormone biosynthetic genes are cytokinin biosynthesis genes.
17. A trait removal construct according to Claims 1,2 or 3 wherein the recombinase coding sequences and 3' region R1 and R2 are independently selected from the group consisting of Cre and Flp.
- 10 18. A trait removal construct according to Claims 1, 2 or 3 wherein the first and second recombinase sites are independently selected from the group consisting of Lox and Frt.
19. A trait removal construct according to Claims 1, 2 or 3 wherein the first, second and third recombinase elements may be genetically linked or
- 15 19. A trait removal construct according to Claims 1, 2 or 3 wherein the first, second and third recombinase elements may be genetically linked or unlinked.
20. A trait removal construct according to Claim 19 wherein the first, second and third recombinase elements may be genetically unlinked and reside in different plants.
21. A method for removing a transgene encoding a genetic trait from a
- 20 21. A method for removing a transgene encoding a genetic trait from a plant cell comprising introducing into the plant cell a trait removal construct of Claims 1, 2 or 3 wherein expression of the recombinase results in excision of any element contained between the recombinase site responsive to the recombinase.
22. A method according to Claim 21 wherein P1 and P2 are selected from the group consisting of:
- 25 a) constitutive plant promoters;
- b) plant tissue-specific promoters;
- c) plant development-stage-specific promoters;
- d) inducible promoters; and
- e) viral promoters.
- 30 23. A method according to Claim 21 wherein P1 is selected from the group consisting of:
- a) male germline promoters;
- b) female germline promoters;
- c) common germline promoters;
- 35 d) flower promoters;
- e) vegetative shoot apical meristem promoters; and
- f) floral shoot apical meristem promoters.

24. A method according to Claim 23 wherein P1 is a male germline promoter and the transgene is excised only from the male germline cells to result in pollen without the transgene.

5 25. A method according to Claim 23 wherein P1 is a female germline and the transgene is excised only from the female germline cells to result in the egg cell without the transgene.

26. A method according to Claim 23 wherein P1 is a common germline promoter and the transgene is excised from both male and female germline cells to result in progeny seeds without the transgene.

10 27. A method according to Claim 23 wherein P1 is a floral SAM or flower promoter.

28. A method according to Claim 23 wherein P1 is a vegetative SAM promoter such that the transgene is excised from germline and somatic cells or tissues derived from the vegetative SAM.

15 29. A method according to Claim 22 wherein P1 is an inducible promoter responsive to heat shock.

30. A method according to Claim 22 wherein P1 is an inducible promoter responsive to a chemical safener.

20 31. A method according to Claim 21 wherein the first, second recombinase and third elements are genetically unlinked and reside in different plants.

25 32. A method for removing a transgene encoding a genetic trait from a plant cell comprising introducing into the plant cell a trait removal construct of Claim 3 wherein expression of the recombinase results in excision of any element contained between the recombinase site responsive to the recombinase.

33. A method according to Claim 21 wherein TG is a transgene selected from the group consisting of:

- 30
- a) genes encoding a transformation marker;
 - b) genes encoding a morphological trait;
 - c) genes conveying sterility;
 - d) genes conveying specific phenotype on a plant or plant cell; and
 - e) hormone biosynthetic genes.

34. A method according to Claim 33 wherein the transgene is selected from the group consisting of:

- 35
- a) antibiotic resistance genes;
 - b) herbicide resistance genes;
 - c) green fluorescent protein genes;
 - d) luciferase genes;

- e) tumorigenic genes;
- f) root inducing genes; and
- g) selectable genes.

35. A method according to Claim 34 wherein the transgene is selected
5 from the group consisting of:

- a) IPT;
- b) Ti plasmid genes; and
- c) Ri plasmid genes.

36. A method for conditionally activating a transgene in a second
10 generation plant comprising:

- 1) providing a construct comprising:
 - a) a first recombinase element having the general structure P1-R1;
 - b) a second recombinase element having general structures
15 P2-RS1-STP-RS1-R2;
 - c) a third recombinase element having the general structure P3-RS2-STP-RS2-TG;

wherein:

- (i) P1 is a first promoter;
- 20 (ii) R1 is a first recombinase coding sequence and 3' region;
- (iii) RS1 is a first recombinase site responsive to a first recombinase;
- (iv) P2 is a second promoter,
- (v) RS2 is a second recombinase site responsive to a second
25 recombinase;
- (vi) STP is a stop fragment;
- (vii) R2 is a second recombinase coding sequence and 3' region
- (viii) TG is a transgene sequence and 3' region ; and
- 30 (vi) P3 is a third promoter;

wherein P1, P2 and P3 are operably linked to their down stream elements;

- 2) providing a transgenic plant comprising the first, second and third recombinase elements;
- 3) activating P1 such that the R1 recombinase coding sequence is
35 expressed in the common germline of a first generation plant, wherein expression of R1 excises the stop fragment from the second recombinase element;

- 4) activating P2 such that R2 is expressed either in the common
germline of the first generation plant or in the second generation
plant, wherein expression of R2 excises the stop fragment from
the third recombinase element allowing expression of the
transgene in the second and all subsequent generations of plants.

37. A method for conditionally activating a transgene in a second
generation plant comprising:

- 1) providing a construct comprising:
- a) a first recombinase element having the general structure
P1-R1;
 - b) a second recombinase element having general structures
P2-RS1-STP-RS1-R2;
 - c) a third recombinase element having the general structure
P3-RS2-STP-RS2-TG;

wherein:

- (i) P1 is a first promoter;
- (ii) R1 is a first recombinase coding sequence and 3' region;
- (iii) RS1 is a first recombinase site responsive to a first
recombinase;
- (iv) P2 is a second promoter,
- (v) RS2 is a second recombinase site responsive to a second
recombinase;
- (vi) STP is a stop fragment;
- (vii) R2 is a second recombinase coding sequence and 3'
region;
- (viii) TG is a transgene sequence and 3' region ; and
- (vi) P3 is a third promoter;

wherein P1, P2 and P3 are operably linked to their down stream elements;

- 2) providing a transgenic plant comprising the third recombinase
element;
- 3) transforming the transgenic plant of (2) with either the second
recombinase element to generate a first plant or the third
recombinase element to generate a second plant;
- 4) crossing the first and second plants such that expression of R1
under the control of P1 in the common germline of the first
generation, excises the stop fragment from the second
recombinase element allowing expressing of R2 under the
control of P2 which in turn, excises the stop fragment from the

third recombinase element, permitting expression of the trait gene(s) under the control of P3 in the second and subsequent generation(s).

38. A method for conditionally activating a transgene in a second generation plant comprising:

1) providing a construct comprising:

- a) a first recombinase element having the general structure P1-R1;
- b) a second recombinase element having general structures P2-RS1-STP-RS1-R2;
- c) a third recombinase element having the general structure P3-RS2-STP-RS2-TG;

wherein:

- (i) P1 is a first promoter;
- (ii) R1 is a first recombinase coding sequence and 3' region;
- (iii) RS1 is a first recombinase site responsive to a first recombinase;
- (iv) P2 is a second promoter,
- (v) RS2 is a second recombinase site responsive to a second recombinase;
- (vi) STP is a stop fragment;
- (vii) R2 is a second recombinase coding sequence and 3' region;
- (viii) TG is a transgene sequence and 3' region; and
- (vi) P3 is a third promoter;

wherein P1, P2 and P3 are operably linked to their down stream elements and wherein, P1 is a germline promoter that can be induced in the first generation of plant life cycle;

2) providing a transgenic plant comprising the first, second and third recombinase elements;

3) inducing the first promoter such that expression of R1 under the control P1 in the common germline of the first generation, excises the stop fragment from the second recombinase element allowing expressing of R2 under the control of P2, which, in turn, excises the stop fragment from the third recombinase element, permitting expression of the trait gene(s) under the control of P3 promoter in in the second and subsequent generation(s).

39. A method for conditionally activating a transgene in a second generation plant comprising:

- 1) providing a construct comprising:
 - a) a first recombinase element having the general structure P1-R1;
 - b) a second recombinase element having general structures P2-RS1-STP-RS1-R2;
 - c) a third recombinase element is selected from the group consisting of the general structures P3-RS2-STP-RS2-TG1 and P4-RS2-STP-RS2-TG2;

wherein:

- (i) P1 is a first promoter;
- (ii) R1 is a first recombinase coding sequence and 3' region;
- (iii) RS1 is a first recombinase site responsive to a first recombinase;
- (iv) P2 is a second promoter;
- (v) RS2 is a second recombinase site responsive to a second recombinase;
- (vi) STP is a stop fragment;
- (vii) R2 is a second recombinase coding sequence and 3' region;
- (viii) TG1 is a first transgene sequence and 3' region;
- (ix) TG2 is a second transgene sequence and 3' region;
- (x) P3 is a third promoter; and
- (x) P4 is a fourth promoter;

wherein P1, P2, P3 and P4 are operably linked to their down stream elements and wherein TG1 and TG2 are different trait transgenes and wherein P3 and P4 are activated in a second generation plant;

- 2) providing a first plant comprising the first and third recombinase elements;
- 3) providing a second plant comprising the second and third recombinase elements;
- 4) crossing the first and second plants to produce a first generation plant wherein conditional expression of the first recombinase coding sequence (R1) under the control of the P1 promoter in the common germline of the first generation, excises the stop fragment from the second recombinase element allowing expressing of the second recombinase coding sequence and

5 '3' region (R2) under the control of P2 promoter, which recombinate, in turn, excises the stop fragments from the two third recombine elements, permitting expression of the trait gene(s) TG1 and TG2 under the control of P3 and P4 promoter, respectively, in the second generation.

40. A method according to Claim 39 wherein, TG1 is a trait gene, TG2 is a lethal gene that blocks plant development, and the third promoter (P3) is expressed earlier in the plant life cycle than the fourth promoter (P4) in the second generation.

10 41. A method according to Claim 39 wherein TG1 is a trait gene, TG2 is a sterility gene that prevents pollen formation and seed set, and the third promoter (P3) is expressed earlier than P4 in the next generation

42. A method according to Claim 36 wherein the first promoter is a constitutive and common germline promoter, and the second promoter is floral common germline promoter, and the third promoter is seed-specific; wherein conditional expression of the first recombine coding sequence in common germline under the control of the first promoter results in expression of the second recombine coding sequence under the control of the second promoter in floral common germline, and wherein expression of the second recombine results in the expression of the trait in the progeny seed.

20 43. A method according to Claim 37 where the first promoter is inducible and responsive to an inducing agent.

44. A method for conditional and transient expression of a trait transgene comprising:

- 25 1) providing a construct comprising:
- a) a first recombine element having the general structure P1-R1;
 - b) a second recombine element having the general structure S2-P2- RS1-STP-RS1-TG-RS2 ;
 - 30 c) a third recombine element having the general structure P3-RS1-STP-RS1-R2

wherein:

- (i) R1 is a first recombine coding sequence and 3' region;
- (ii) RS1 is a first recombine site responsive to a first recombine;
- 35 (iii) R2 is a second recombine coding sequence and 3' region;

- (iv) RS2 is a second recombinase site responsive to a second recombinase
- (iv) STP is a stop fragment;
- (v) TG is trait transgene sequence and 3' region;
- 5 (vi) P1 is a first promoter which will be activated in the germline of the first generation;
- (vii) P2 is a second promoter which will be activated in somatic and/or germline cells and may be activated prior to or at the same time as P3;
- 10 (viii) P3 is a third promoter which is activated in the germline;

wherein P1, P2 and P3 are operably linked to their down stream elements, and

- 2) providing a first generation transgenic plant comprising all three recombinase elements;
- 15 3) activating the first promoter (P1) in the first generation plant of (2) wherein the first recombinase coding sequence is expressed priming both the second and third recombinase elements;
- 4) activating the second promoter wherein the transgene is expressed; and
- 20 5) activating the third promoter wherein the second recombinase coding sequence is expressed excising the second recombinase element.

45. A method for conditional and transient expression of a trait transgene comprising:

- 25 1) providing a construct comprising:
- a) a first recombinase element having the general structure P1-R1;
- b) a second recombinase element having the general structure RS2-P2- RS1-STP-RS1-TG-RS2 ;
- 30 c) a third recombinase element having the general structure P3-RS1-STP-RS1-R2

wherein:

- (i) R1 is a first recombinase coding sequence and 3' region;
- (ii) RS1 is a first recombinase site responsive to a first recombinase;
- 35 (iii) R2 is a second recombinase coding sequence and 3' region;

- (iv) RS2 is a second recombinase site responsive to a second recombinase
- (iv) STP is a stop fragment;
- (v) TG is trait transgene sequence and 3' region;
- 5 (vi) P1 is a first promoter which will be activated in the germline of the first generation;
- (vii) P2 is a second promoter which will be activated in somatic and/or germline cells and may be activated prior to or at the same time as P3;
- 10 (viii) P3 is a third promoter which is activated in the germline;
- wherein P1, P2 and P3 are operably linked to their down stream elements, and
- 2) providing a first plant comprising the first recombinase element;
 - 3) providing a second plant comprising the second and third
 - 15 recombinase elements, wherein the second plant is made homozygous for the elements;
 - 4) crossing the first and second plants to produce a first generation plant wherein the first promoter (P1) is activated, expressing the
 - 20 first recombinase coding sequence (R1) which excises the stop fragments from the second and third recombinase elements sequentially activating the trait (TG) gene and the second (R2) recombinase coding sequence, which in turn removes the second recombinase element from the chromosome, permitting transient expression of the transgene.
- 25 46. A method according to Claims 44 wherein P1 is a first germline promoter that is induced in the first generation of plant life cycle; and wherein all three recombinase elements are introduced into a plant either by co-transformation, sequential transformations, or genetic crosses after single transformation in a plant.
- 30 47. A method according to any one of Claims 44-46 wherein the first promoter (P1) is selected from the group consisting of constitutive promoter, seed promoter, a vegetative or floral shoot apical meristem promoter, and floral common germline promoter.
- 35 48. A method according to Claim 47 wherein the P3 promoter is male germline-specific promoter such that the TG is removed from only the pollen of the first generation.

49. A method according to Claim 47 wherein the P3 promoter is expressed in the floral common germline to result in TG removal from both pollen and the progeny seed of the first generation.

50. A method according to Claim 44 wherein:

- 5 a) P1 is a floral common germline promoter that expresses the first recombinase coding sequence (R1) in the common germline of the first generation and is not expressed in seed or germinating seed;
- b) P2 expresses the transgene (TG) in the seed of the second generation; and
- 10 c) P3 expresses the second recombinase coding sequence (R2) in the common germline of germinating seeds of the second generation to result in the transgene (TG) removal from both pollen and the seed of the second generation.

15 51. A method for conditional expression of male sterility in the first generation plant comprising:

- 1) providing a construct comprising:
 - a) a first recombinase element having the general structure P1-R1;
 - 20 b) a second recombinase element having the general structure RS2-P2- RS1-STP-RS1-TG-RS2 ;
 - c) a third recombinase element having the general structure P3-RS1-STP-RS1-R2;

wherein:

- 25 (i) R1 is a first recombinase coding sequence and 3' region;
- (ii) RS1 is a first recombinase site responsive to a first recombinase;
- (iii) R2 is a second recombinase coding sequence and 3' region;
- 30 (iv) RS2 is a second recombinase site responsive to a second recombinase;
- (v) STP is a stop fragment;
- (vi) TG is transgene sequence and 3' region encoding male sterility;
- 35 (vii) P1 promoter is a common germline promoter which is activated in vegetative SAM, floral SAM, or flower of the first generation and is not expressed in seed;

(viii) P2 is a second sporophytic anther or anther primordia promoter;

(ix) P3 is a third seed-specific promoter that is activated in the germline of the second generation and

5 wherein P1, P2 and P3 are operably linked to their down stream elements, and

2) providing a first transgenic plant comprising the first recombinase element;

3) providing a second transgenic plant comprising the second and third recombinase elements in a homozygous state;

10 4) crossing the first and second plants to produce a first generation plant wherein the expression of the first recombinase under the control of the first promoter excises the stop fragment from both the second and third recombinase elements, causing the male sterility transgene to be expressed;

15 5) crossing the first generation plant of step (4) with a male fertile inbred to produce a second generation plant, wherein activation of the seed specific third promoter expresses the second recombinase, excising the male sterility transgene and restoring male fertility.

20 52. A method for expressing conditional male sterility in the first and second generations comprising:

1) providing a construct comprising:

a) a first recombinase element having the general structure P1-R1 ;

25 b) a second recombinase element having the general structure P2- RS1-STP-RS1-MS ;

c) a third recombinase element having the general structure P2-RS2-STP-RS2-MS;

30 d) a fourth recombinase element having the general structure P1-R2;

wherein:

(i) R1 is a first recombinase;

(ii) RS1 is a first recombinase site responsive to a first recombinase

35 (iii) R2 is a second recombinase;

(iv) RS2 is a second recombinase site responsive to a second recombinase;

(v) STP is a stop fragment;

- (vi) TG is a transgene encoding male sterility with 3' region;
and
(vii) P1 is germline promoter that is activated in the plant
life cycle earlier than, or at the same time as, P2 and is
selected from the group consisting of; a constitutive
promoter, seed-specific promoter, and a vegetative shoot
apical meristem promoter;
(viii) P2 is a promoter which expresses the transgene,

wherein P1 and P2 are operably linked to their down stream elements;

- 2) providing a first plant comprising the first and third recombinase elements;
- 3) providing a second plant comprising the second and third recombinase elements;
- 4) providing a third plant comprising the fourth recombinase element;
- 5) crossing the first and second plants to obtain first progeny in which expression of the first recombinase under the control of the P1 promoter of the first recombinase element excises the stop fragment from the second recombinase element, allowing the expression of the transgene encoding male sterility;
- 6) crossing the progeny of step (5) with the third plant to obtain second progeny in which expression of the second recombinase under the control of the P1 promoter in the fourth recombinase element excises the stop fragment from the third recombinase element, allowing the expression of the male sterility gene.

53. A method according to Claim 49 wherein the P2 promoter is an anther- or anther primordia-specific promoter that is selected from the group consisting of a tapetum-specific promoter, and a male germline-specific promoter.

54. A method for expressing conditional male sterility in a plant comprising:

- 1) providing constructs comprising:
 - a) a first recombinase element having the general structure
P1-R1 ;
 - b) a second recombinase element having the general structure
RS2-P2- RS1-STP-RS1-TG -RS2-SG;
 - c) a third recombinase element having the general structure
P1-R2 ;

wherein:

- (i) R1 is a first recombinase;
(ii) RS1 is a first recombinase site responsive to a first recombinase;
(iii) RS2 is a second recombinase site responsive to a second recombinase;
(iv) STP is a stop fragment;
(v) SG is a plant selectable gene; and
(vi) TG is a transgene sequence and 3' region encoding male sterility;
(vii) P1 is a common germline promoter that is activated in the plant life cycle earlier than or at the same time as P2 promoter and is selected from the group consisting of; a constitutive promoter, seed-specific promoter, and a vegetative or floral shoot apical meristem promoter;
(viii) P2 is an anther- or anther primordia-specific promoter that is activated in the life cycle at the same time as or later than P1 promoter and is selected from the group consisting of a tapetum-specific promoter, male germline-specific promoter;
- wherein P1, and P2 are operably linked to their down stream elements;
- 2) providing a first plant comprising the first recombinase element;
 - 3) providing a second plant comprising the second recombinase element;
 - 4) providing a third plant comprising the third recombinase element;
 - 5) crossing the first and second plants to produce progeny in which expression of the first recombinase under the control of P1 promoter excises the stop fragment from the second recombinase element permitting the expression of the male sterility gene under the control of the P2 promoter;
 - 6) crossing the progeny of step (5) with a male fertile plant to produce progeny seeds;
 - 7) selecting progeny seeds of step (6) on the basis of the gene product of the selectable gene which ensures male sterility; and
 - 8) crossing the male sterile progeny of step (7) with the third plant of step (4) to produce a progeny in which expression of the second recombinase under the control of the P1 promoter

excises the male sterility trait from the third recombinase element restoring male fertility in the hybrid progeny.

55. A method for transgene removal comprising:

- 1) providing a construct comprising:
 - a) a first recombinase element having the general structure P1-R1;
 - b) a second recombinase element having the general structure RS1-P2-TG-RS1 ;

wherein:

- (i) R1 is a first recombinase coding sequence and 3' region;
- (ii) RS1 is a first recombinase site responsive to a first recombinase;
- (iii) TG is transgene sequence and 3' region encoding either a trait or a transformation marker;
- (iv) P1 is a first promoter that expresses R1 in the germline of the first generation;
- (vi) P2 is a second promoter, wherein P2 is activated before or at the same as P1;

wherein P1, and P2 are operably linked to their down stream elements;

- 2) providing a first plant comprising the first recombinase element;
- 3) providing a second plant comprising the second recombinase element;
- 4) crossing the first and second plants to create a first generation plant wherein the first promoter (P2) is activated, expressing the transgene;
- 5) activating the first promoter (P1) wherein the recombinase is expressed, resulting in the removal of the transgene from the chromosome.

56. A method according to Claim 55 wherein P1 is a common germline selected from the group consisting of *Apetala 3*, *Pistillata*, *Bcp 1*, *Apetala 1*, *Leafy*, and *Agamous* genes .

57. A method according to Claim 55 wherein P1 is a male germline promoter which excises the transgene from the pollen.

58. A method according to Claim 55 wherein P1 is a floral common germline promoter that expresses the recombinase to excises the transgene from the progeny seed.

59. A method according to Claim 57 wherein P1 is a promoter derived from *Arabidopsis* selected from the group consisting of *Apetala 3* (AP3), *Pistillata* (P1), *Bcp 1* genes or a synthetic anther promoter.

60. A method according to Claim 58 wherein P1 is a promoter derived from *Arabidopsis* selected from the group consisting of *Apetala 3* (AP3), *Pistillata* (P1), *Leafy*, *Agamous*, *Apetala 1*.

61. A method for transformation marker excision comprising:

1) providing a construct comprising a recombinase element having the general structure RS1-P1-R1-P2-TG1-RS1 P3-TG2;

wherein:

(i) R1 is a recombinase coding sequence and 3' region;

(ii) RS1 is a recombinase site responsive to recombinase R1;

(iii) TG1 is transgene sequence and 3' region encoding a transformation marker and 3' region;

(iv) TG2 is trait transgene sequence and 3' region;

(v) P1 is a first promoter that is activated in the germline of the primary transformant;

(vi) P2 is a second promoter that is activated in the primary transformant;

(vii) P3 is a third promoter;

wherein P1, P2 and P3 are operably linked to their down stream elements;

2) providing a transgenic plant comprising the recombinase element;

3) activating the second promoter (P2) whereby the transgene encoding a marker (TG1) expressed;

4) activating the first promoter (P1) whereby the recombinase (R1) is expressed resulting in the excision of the transgene encoding a marker (TG1); and

5) activating the third promoter (P3) whereby the trait transgene (TG2) is expressed;

62. A method according to Claim 61, wherein P1 is a common germline promoter that is inducible.

63. A method according to Claim 61, wherein P1 is a common germline promoter from *Arabidopsis* heat shock promoter.

64. A method according to Claim 61, wherein P1 is a common germline promoter from safener inducible promoter IN-2.

65. A method according to Claim 61, wherein P1 is a common germline promoter from developmentally regulated genes of shoot apical meristem, floral germline, or male germline cells.

5 66. A method according to Claim 61, wherein P1 is a common germline promoter derived from vegetative and floral shoot apical meristem, flower, or male germline wherein the vegetative and floral shoot apical meristem promoter is selected from the group consisting of *Leafy*, *Apetala 1*, *Apetala 3* (AP3), *Pistillata* (PI) or their orthologs from other species, the flower promoter is selected from the group consisting of synthetic anther promoter and Bcp I genes or their orthologs
10 from other species, and the male germline promoter is selected from the group consisting of *Apetala 3* (AP3), *Pistillata* (PI) or their orthologs from other species.

67. A method according to Claim 57, wherein the transformation marker is a morphological trait, hormone biosynthetic gene, selectable gene.

15 68. A method for the conditional gametophytic expression of male sterility comprising:

- 1) providing a construct comprising:
 - a) a first recombinase element having the general structure P1-R1;
 - b) a second recombinase element having the general structure P1-RS1-STP-RS1-R2; and
 - 20 c) a third recombinase element having the general structure P2-RS2-STP-RS2-TG1;
 - d) a linked gene construct having the general formula, TG2-TG3;

25 wherein:

- (i) P1 is a first promoter;
- (ii) R1 is a first recombinase coding sequence and 3' region;
- (iii) RS1 is a first recombinase sequence responsive to a first recombinase;
- 30 (iv) P2 is a second pollen specific promoter,
- (v) RS2 is a second recombinase sequence responsive to a second recombinase;
- (vi) STP is a stop fragment;
- (vii) R2 is a second recombinase coding sequence and
35 3' region;
- (viii) TG1 is a transgene sequence and 3' region encoding gametophytic male sterility;

(ix) TG2 is a transgene sequence and 3' region encoding a plant trait;

(x) TG3 is a gametophytic male fertility restorer gene;

wherein P1 and P2 are operably linked to their down stream elements;

- 5 2) providing a transgenic plant comprising the construct of step (1);
- 3) activating the first promoter (P1) whereby the recombinase (R1) is expressed resulting in the excision of the stop fragment (STP) from the second recombinase element, expression of the second recombinase and excision of the stop fragment (STP) from the
- 10 third recombinase element;
- 4) activation of the pollen specific promoter (P2) where in the male sterility transgene (TG1) is expressed in the pollen;
- 5) selfing the plant of step (4) wherein TG2 and TG3 are expressed in the resulting second generation plant.

15 69. A method according to Claim 68, wherein TG1 is barnase and TG3 is barstar.

 70. A method for the conditional expression or excision of a transgene in a plant comprising:

- (i) providing a multiplicity of recombinase elements, each
- 20 recombinase element comprising:
- a) at least one promoter;
- b) a DNA fragment;
- wherein the DNA fragment comprises at least one genetic element selected from
- the group consisting of, a recombinase coding sequence, a
- 25 site-specific recombinase sequence responsive to a recombinase; a stop fragment and a transgene;
- (ii) introducing at least two of the recombinase elements of step (i) into at least one plant wherein the at least two recombinase elements are selected from the group consisting of
- 30 a) a recombinase element having a first recombinase under the control of a promoter; and
- b) a recombinase element having a second recombinase under the control of a promoter whose expression is dependent on the expression of the first recombinase;
- 35 (iii) activating at the promoter of step (ii)(a) wherein the expression of the recombinase is effected by the expression of the first recombinase.

71. A method for the developmentally regulated germline expression of a transgene comprising:

- (i) providing a construct comprising a developmentally regulated germline promoter operably linked to a transgene;
- 5 (ii) introducing the construct of step (i) into at least one plant; and
- (iii) activating the promoter whereby the transgene is expressed.

72. A method according to Claim 71, wherein the promoter is activated in the common germline.

73. A method according to Claim 72 wherein the promoter is from a homeotic gene.

74. A method according to Claim 73 wherein the homeotic gene is selected from the group consisting of AP3, PI, LFY, AG, and AP1.

75. A method according to Claim 71, wherein the promoter is activated in the male germline.

76. A method according to Claim 75 wherein the promoter is from a homeotic gene.

77. A method according to Claim 76 wherein the homeotic gene is selected from the group consisting of AP3 and PI.

78. A method according to Claim 75 wherein the promoter is an anther-specific promoter selected from the group consisting of BCP1, SAP and TA29.

79. A method according to Claim 71, wherein the transgene encodes a recombinase.

80. A trait removal construct comprising:

- 25 a) a first recombinase element comprising a first promoter operably linked to a sequence encoding a first recombinase;
- b) a second recombinase element comprising a second promoter, a stop fragment bounded by site specific sequences responsive to the first recombinase and a sequence encoding a second recombinase wherein the presence of the stop fragment inhibits expression of the second recombinase, and wherein the first and second recombinases are different; and
- 30 c) a DNA molecule bounded by site specific sequences responsive to the second recombinase;

wherein expression of the first recombinase excises the stop fragment from the second recombinase element, operably linking the second promoter and the sequence encoding the second recombinase, and wherein expression of the second recombinase results in site specific recombination within the DNA molecule bounded by site specific sequences responsive to the second recombinase.